Rapid extraction of fungal DNA for PCR amplification

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Polymerase chain reaction (PCR) is a technique widely used in fungal research. One of its advantages is the ability to amplify very small amounts of DNA, in the picograms range, even in the presence of diverse contaminants. In spite of this, most of the extraction protocols of fungal DNA are designed for the obtention of microgram amounts of very pure DNA, requiring the establishment of relatively large fungal cultures and long extraction procedures. These protocols are needlessly complicated for PCR experiments. On the other hand, some authors have pointed out the feasibility of using single spores (1) or boiled mycelium (2) as a source of DNA in PCR experiments. This is advantageous for detection purposes, but when working with hundreds of strains in population studies, obtaining the material from the culture plate can be cumbersome and favor contaminations.

Two widely cited methods (1, 3) allow the DNA extraction in Eppendorf tubes, simplifying and reducing the scale of the extraction. Both methods start from lyophilized or fresh mycelium, ground by hand with a mortar and pestle. However, this procedure is cumbersome, and implies careful sterilization of the material and the use of liquid nitrogen.

We have developed a faster and less prone to contamination procedure by culturing the fungus directly in an Eppendorf tube, and suppressing the phenolization step, as suggested in other recent protocol for plant DNA extraction (4). The proposed method is as follows: A 1.5 ml Eppendorf tube is filled with 500 µl of liquid Potato−dextrose medium. The culture is started by inoculating some hyphal threads and allowed to grow for 72 hours at 25°C. The mycelial mat is pelleted by centrifugation for 5 minutes at 13,000 rpm in a microfuge, washed with 500 µl of TE buffer and pelleted again. The TE is decanted and 300 µl of extraction buffer are added. This buffer is the same as that described by Raeder and Broda (3) (200 mM Tris HCl pH 8.5, 250 mM NaCl, 25 mM EDTA, 0.5% SDS). The mycelium is crushed with a conical grinder (Treff AG, Degersheim, Switzerland), fitting exactly the tube and actioned by hand or electric potter at 200 rpm for some minutes. After that, 150 µl of 3 M sodium acetate, ph 5.2 are added, and tubes are placed at −20°C for about ten minutes. Tubes are then centrifuged in a microfuge and the supernatant transferred to another tube. Then, an equal volume of isopropanol is added, and after at least 5 minutes at room temperature, the precipitated DNA is pelleted by centrifugation in a microfuge. After a wash with 70% ethanol, the pellet is vacuum dried for some minutes and resuspended in 50 µl of TE. The amount of DNA obtained in this way, is around 3−6 µg. This is enough for at least 50 PCR reactions, as determined by titration of DNA concentration in several reactions. Alternatively, in case more material is needed, the fungal culture can be made in a 15 ml centrifuge tube. After the mycelium is pelleted and washed in a benchtop centrifuge, it is transferred to an Eppendorf tube and crushed in the way described above. This culture yields about 25−35 µg of DNA, enough for at least 500 PCR reactions.

Fungal DNA obtained in this way, has been used in two PCR experiments. The first one was intended for detection of polymorphisms by random amplified polymorphic DNA (RAPD-PCR) (5) in the phytopathogenic fungi *Fusarium oxysporum* and *Rhizoctonia solani*. This experiment was performed according to the conditions and cycling parameters described by Williams *et al.* (5). The primer used was OPA-2 (Operon Technologies, Alameda, CA), with sequence 5′-CAGGCCCTTC-3′. The second experiment was designed for amplification of a rDNA fragment in the same fungi. In this case, the reaction buffer was composed as before, but primers were 5′-ACCCGCTGAA-CTTAAGC-3′ and 5′-TACTACCACCAAGATCT-3′. Cycling parameters were: 94°C for 1 minute, 50°C for 1 minute and 68°C for 2 minutes, for 35 cycles and a final autoextension step of 72°C for 5 minutes. In all cases, 1 µl of the described fungal miniprep was used as DNA template. Fifteen µl of reaction product were analysed in a 1.4% agarose gel, and visualized by ethidium bromide staining. (Figure 1).

This protocol showed to be highly reproducible. The variation of growth rate of different strains is somewhat compensated by the broad range of DNA concentrations allowed by PCR, producing similar amounts of amplified product with different species and strains. When compared to other protocols of fungal DNA extraction, it suppresses the need of using liquid nitrogen, lyophilized material, phenolization, RNAse treatment and long incubation or centrifugation steps. As a consequence, it allows the processing of dozens of fungal strains by day.

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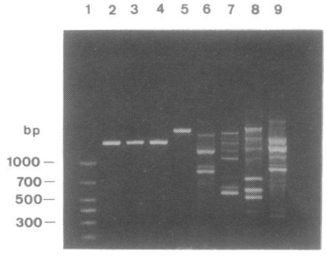

Figure 1. Products of PCR amplification of DNA miniprep. Lane 1, size standard 1000, 700, 500, 400, 300 and 200 bp. Lanes 2−5, rDNA amplified from *Fusarium oxysporum* f.sp. *lycopersici*, *F. o.* f.sp. *radicis-lycopersici*, *Rhizoctonia solani* AG 1 and AG 3, respectively. Lanes 6−9, RAPD product from *F. o.* f.sp. *lycopersici*, *F. o.* f.sp. *radicis-lycopersici*, and *R.solani* (watermelon strain and pine strain), respectively.